

PCT

WORLD INTELLEC
In

INTERNATIONAL APPLICATION PUBLISHED

(51) International Patent Classification⁶ :

F25D 25/00, A01N 1/02

WO 9602801A1

A

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/GB95/01677

(22) International Filing Date: 17 July 1995 (17.07.95)

(30) Priority Data:

9414428.4

16 July 1994 (16.07.94)

GB

(71) Applicant (for all designated States except US): SECRETARY OF STATE FOR FOREIGN AND COMMONWEALTH AFFAIRS [GB/GB]; Overseas Development Administration, 94 Victoria Street, London SW1E 5JL (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): RANA, Krishen, Jugjivan [GB/GB]; 5 Western Drive, Bridge of Allan, Stirlingshire FK9 4AX (GB).

(74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

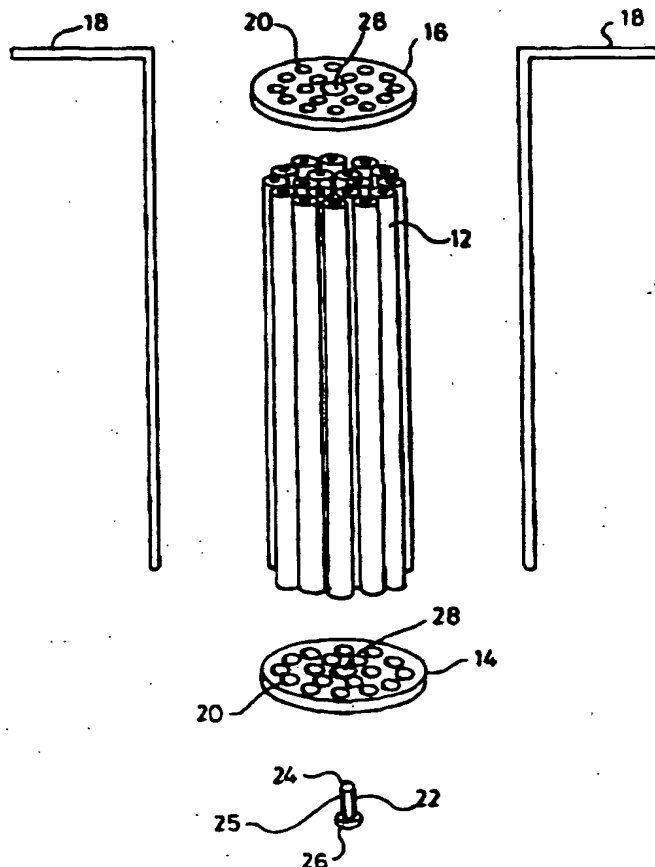
Published

With international search report.

(54) Title: COOLING ASSEMBLY FOR CRYOPRESERVATION

(57) Abstract

Cooling assembly (10) for use in a cryopreservation cooler, comprises: a plurality of individual sample holders (12), each sample holder (12) being capable of accepting a sample receptacle (13) containing a sample to be preserved; and sample holder retaining means (14, 16) for retaining the sample holders (12) in a spaced-apart relationship, such that a coolant fluid may pass between the sample holders (12). Coolers comprising the cooling assembly defined above, and methods of cooling samples.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Title: COOLING ASSEMBLY FOR CRYOPRESERVATION

Field of the Invention

This invention relates to apparatus for and methods of cryopreservation.

Background of the Invention

Cryopreservation is the process whereby material, particularly biological material, suspended in an appropriate medium (typically, buffered saline) containing a cryoprotectant, is cooled to a low temperature so as to preserve the sample. It has been widely acknowledged (and published) since the 1950s that, for biological material, the cooling rate the biological material is subjected to is critical to successful cryopreservation, and that reproducibility of the desired optimal cooling rates has a significant bearing on the overall success of the cryopreservation process. It has generally been found that a linear cooling rate (i.e. a substantially constant fall in temperature per unit time) over the entire cooling phase is most desirable. However, a certain degree of departure from such a linear rate inevitably occurs during freezing of the sample, due to the transition of the sample from an essentially liquid phase to the solid phase.

Just about any biological material may be cryopreserved, but typically the process is applied to spermatozoa, eggs or embryos. As will be apparent, the process is frequently used in animal husbandry, but may also be applied to horticulture or any field which can require the preservation of biological material. Under current practices, sperm or egg samples are typically packaged in synthetic plastics straws (0.25 - 5ml in volume) and cooled using varying methods. Following cooling to the desired sub-zero temperature, the straws are removed and plunged into liquid nitrogen ("LN") and then transferred to canisters for storage in an LN Dewar vessel until required. The plunging of cooled samples into LN is a process called "purging". Sudden reduction in temperature by such purging (after the initial cooling at a constant rate) is found desirable to achieve optimal viability in the stored material. Performing the purging step can sometimes present a problem, in that there is typically a time delay between cooling the samples in a cooler

and then removing them from the cooler and plunging them into LN provided in a second LN vessel. Although the time delay is normally quite short, the cooled samples can start to warm very rapidly because their temperature is so much lower than the ambient temperature outside the cooler. This applies, in particular, to cryopreservation under field conditions, especially in hot climates.

Conventional coolers may typically be classified into two groups: controllable rate coolers ("CRCs") or "passive" coolers. The optimal linear cooling rates can only be achieved by the use of CRCs. These are expensive and comparatively complex pieces of apparatus, in which the rate of cooling is determined by expensive electronic equipment which controls the rate at which LN coolant is pumped from an LN reservoir into a cooling chamber containing the sample to be frozen.

Passive coolers typically comprise a Dewar vessel containing a static reservoir of LN. The LN vessel has an opening through which samples are introduced, the opening defining the upper end of a neck region. The lower end of the neck region is defined by a more or less pronounced shoulder. Use of such coolers generally involves the suspension of samples at a pre-determined height above the LN reservoir, the cooling being achieved by vapour from the LN. This arrangement has the advantage of simplicity of construction and comparative cheapness. The simplicity of passive coolers means that they can be readily portable, and may thus be used under field conditions (for several days if need be). However, in such coolers the cooling rates are dependent on the difference between ambient (LN vapour) and sample temperatures. Since LN vapour temperature is low (-100°C), when the samples are introduced into the cooler they cool rapidly. After cooling has begun, the temperature difference between the LN vapour coolant and the samples is reduced and the subsequent cooling rate therefore is typically lower than the initial cooling rate. This is readily apparent from the pre-freeze and post-freeze cooling rates. Accordingly, optimal linear cooling rates are not currently obtainable using such passive coolers.

Moreover, results obtained with conventional passive coolers are not reproducible. Cooling rates may vary both between individual samples cooled in a single batch and

between different batches. This lack of reproducibility is a considerable problem, as it prevents cryopreservation of different samples with a predictable degree of viability, which in turn leads to uncertainty when samples are retrieved from storage. As a result, frequently more stored material will be used than may be strictly necessary, in order to ensure that at least some of the material used is viable. This, in turn, means that more material must be subjected to cryopreservation to begin with.

Several approaches have been used to achieve the desired cooling rates during cryopreservation. The accuracy and reproducibility of the cooling rate depend on the system used. Prior to the use of controlled rate coolers (CRCs) biological samples were cooled either in alcohol baths or more simply on a rack mounted at a predetermined distance above liquid nitrogen or as sperm pellets on dry ice blocks. Another sub-optimal method is the positioning of samples in holes drilled in a solid aluminium block, which is then immersed in LN.

Other methods of cryopreservation have also been attempted, especially under field conditions away from a laboratory. For example, dry ice blocks have been used but these have a very short life span (1-2 days) and this can limit the range of field cryopreservation. Moreover, the cooling rates during the cryopreservation are not linear, and there is a complete lack of reproducibility in the results obtained under such conditions.

Thus, there has not so far been developed a cheap, simple cooler which is capable of achieving a reproducible, linear cooling rate. In particular no such device is available for use under field conditions.

Summary of the Invention

In a first aspect the invention provides a cooling assembly for use in a cryopreservation cooler, the assembly comprising: a plurality of individual sample holders, each sample holder being capable of accepting a sample receptacle containing a sample to be preserved; and sample holder retaining means for retaining the sample holders in a spaced apart relationship, such that a coolant fluid may pass between the sample holders.

Typically the cooling assembly is used with a conventional cryopreservation cooler, preferably a passive cooler. Conveniently the coolant fluid will be liquid nitrogen (LN) vapour from a static reservoir of LN provided in the cooler vessel. Desirably, the cooling assembly is held, in use, in the neck region of the cooler.

Preferably the cooling assembly is provided with an assembly support means, such as arms or a flange, to suspend the cooling assembly from the opening of the LN cooler vessel in the neck region thereof. In a preferred embodiment the cooling assembly support means may make releasable engagement with a collar (preferably adjustable) provided on the outside of the cooler. Typically the cooling assembly support means comprises two support arms, one either side of the cooling assembly which extend above the assembly substantially parallel to the long axis thereof. The arms preferably comprise a portion which projects radially outwards, which projecting portion releasably engages an accommodating slot provided in the collar. Desirably, adjustment of the collar in one manner allows for vertical movement of the cooling assembly relative to the cooler, and adjustment of the collar in another manner varies the diameter thereof, such that the collar may be fitted to coolers of different sizes.

It is found that use of the cooling assembly defined above, in conjunction even with a passive cooler, is capable of obtaining a substantially linear rate of cooling of samples. Furthermore, the ability of the coolant to pass freely among the individual sample holders greatly reduces variation of results between samples in a single run. Use of the cooling assembly is found to give highly reproducible results.

Preferably the sample holders are arranged substantially vertically within the cooling assembly. Desirably the sample holders are substantially tubular, each sample holder being capable of accepting a substantially tubular sample receptacle, such as a straw, of the type commonly used in cryopreservation. Advantageously, the sample holders are made of material with a high thermal conductivity (such as copper, aluminium or brass). Typically the cooling assembly will comprise between 10 and 80 sample holders, particularly between 12 and 70 sample holders.

Preferably the arrangement will be such that the straws or other receptacles containing the samples are thermally insulated from the sample holders to a small degree (compared to the intimate thermal contact provided between the coolant fluid and the external surface of the sample holders). Desirably the small degree of thermal insulation is afforded by a thin air-jacket around each sample receptacle. The purpose of providing a small degree of thermal insulation between the sample receptacle and the sample holder is to prevent the establishment of a temperature gradient along the sample receptacle, and so to provide for more uniform cooling.

In a preferred embodiment, samples are placed in receptacles for cryopreservation, each receptacle being supported by sample receptacle support means which prevents the receptacle from making substantial contact with the wall of the sample holder, thereby forming a thin air-jacket around each sample receptacle. Conveniently the receptacle is a straw (e.g. reference numeral 13, Figure 4) of the conventional type, the sample receptacle support means comprising a projection located at the bottom of a tubular support holder, the projection being of appropriate dimensions as to become inserted a short distance into the straw, thereby preventing the straw from touching the bottom or sides of the sample holder. Desirably, the sample receptacle support means comprises a material of comparatively poor thermal conductivity, such as PVC, which helps to maintain a small degree of thermal insulation between the sample receptacle and the sample holder.

Conveniently the sample holder retaining means comprises a base plate, with a plurality of holes drilled therethrough, each hole serving to receive and retain a respective sample holder. The spacing between the holes in the base plate thus determines the separation between sample holders. Typically the sample holder retaining means will further comprise a top plate, of similar character to the base plate. The top plate will typically comprise a plurality of holes, corresponding to the holes drilled through the base plate. The sample receptacle, when positioned in a respective sample holder, will desirably project through the holes in the top plate (to facilitate manipulation of the sample receptacles). The top plate will thus, in preferred embodiments, serve to support the upper end of the sample receptacles and help to prevent them from touching the sides of

the sample holders.

Preferably the cooling assembly support arms will be rigidly connected (e.g. welded) to the base plate, and preferably also to the top plate, thereby providing considerable strength to the assembly. Advantageously the receptacle support means may also serve to hold the cooling assembly together and thus provide additional strength. Conveniently the receptacle support means may be positioned so as to pass through the hole in the base plate, from the underside, and then engage the sample holder in a screw threaded engagement so as to assist in holding the cooling assembly together and retaining the sample holder in position. Where both the base and top plates are rigidly connected to the support arms, the separation between the base plate and the top plate is preferably such as to allow for removal of the sample holders from the assembly, if desired, by removal of the sample receptacle support means.

Desirably, a number of top plates may be used in conjunction with the cooling assembly. For example, in one embodiment the top plate welded to the cooling assembly support arms is provided with lugs or holes which may be engaged by corresponding projections provided on one or more different "adaptor" top plates. The adaptor plates are substantially similar, but the holes drilled therethrough will be of a different diameter in different adaptor plates, such that different adaptor plates will be intended for use with sample receptacles of different sizes.

Advantageously the cooling assembly will be formed with a channel therethrough or form such as a channel in use, the channel being of sufficient dimensions to permit the passage of a sample receptacle. Preferably the cooling assembly will be provided with such a channel, but as an alternative, the channel may be formed, in use, between the cooling assembly and the inner wall of the cooler vessel.

In a particular embodiment, the top and base plates of the cooling assembly are both equipped with a central aperture, which apertures are aligned, a channel being defined between the apertures by the surrounding sample holders.

An advantage of this arrangement is that, once cooled to the desired temperature, the sample receptacles can be retrieved from the cooling assembly and passed through the channel into a storage canister positioned beneath the cooling assembly, in or above the LN reservoir. Clearly, it would be possible to provide a plurality of such channels, (e.g. one for each sample holder), if desired. Thus the same cooler can be used for both cooling and storage of samples, without requiring the cooling assembly to be moved prior to placement of cooled samples in a storage canister. This arrangement allows the samples to be purged without requiring a second LN vessel in which to plunge the samples. Moreover, the cooled samples are in contact with the LN vapour at nearly all times, so that the samples cannot start to warm up in the period between cooling and purging. This is particularly important when attempting to cryopreserve material under field conditions in hot (e.g. tropical) climates.

An optional additional feature is the provision of sample receptacle delivery means, which serves to guide the cooled sample receptacles into the storage canister. The sample receptacle delivery means may comprise an elongate member, locatable in the channel formed in the cooling assembly (or in a channel formed between the cooling assembly and the cooler vessel), and preferably extending into the storage canister positioned beneath, thereby guiding sample receptacles, placed in the channel, into the storage canister.

Typically, several such storage canisters are held in the waist of an LN vessel. Thus, when a cooling run has been completed, a storage canister can be manoeuvred from the side of the LN vessel to a position beneath the cooling assembly to receive the sample receptacles. The sample receptacle delivery means is located in the channel in the assembly so as to extend in to the storage canister. When the sample receptacles have been placed in the canister, the sample receptacle delivery means is removed and the canister may be re-positioned at the side of the vessel. If further samples are to be cooled, another storage canister can be positioned beneath the cooling assembly to accept these when cooled to the correct temperature, and the sample receptacle delivery means replaced. In this way an LN vessel can perform the functions of both cooling and storing samples simultaneously.

Storage canisters are typically provided with a long thin handle, extending through the neck region and out of the opening of the LN vessel in which they are positioned, which handles facilitate manipulation of the canisters. Accordingly, in a preferred embodiment, the collar provided on the outside of the cooler will desirably comprise a plurality of slots, vertically disposed around its circumference, to accommodate the storage canister handles.

In a second aspect the invention provides a cryopreservation cooler, comprising the cooling assembly defined above. Preferably the cooler is a passive cooler comprising a static reservoir of LN and is capable of achieving a substantially linear cooling rate. Desirably the cooler can perform the functions of both cooling and storing samples simultaneously. Further, the cooler is able to give highly reproducible results.

In a third aspect the invention provides a method of subjecting material to a substantially linear cooling rate in a passive cryopreservation cooler, the method comprising use of the cooling assembly defined above.

The invention will now be further described by way of illustrative example and with reference to the drawings, in which:

Figure 1a shows a side elevation of an embodiment of the cooling assembly of the invention;

Figure 1b shows the same embodiment in plan view;

Figure 1c shows, in longitudinal section, to a different scale, a passive cryopreservation cooler comprising the cooling assembly of the invention;

Figure 2 shows an exploded perspective view of the cooling assembly shown in Figure 1c and to the same scale thereof;

Figure 3 shows, to a different scale, an exploded perspective view of a similar embodiment of the cooling assembly of the invention;

Figure 4 shows an enlarged longitudinal sectional view of a sample receptacle in a sample holder, together with sample receptacle support means, and part of the sample holder retaining means;

Figure 5 shows a collar for use in suspending the cooling assembly of the invention in an LN vessel;

Figures 6a - 6c show, for information, graphs of cooling rate (temperature against time) obtained for samples cooled in a controlled rate cooler (a), a passive cooler comprising a cooling assembly in accordance with the invention (b) and a passive cooler used without the cooling assembly of the invention (c); and

Figure 7 shows graphs of cooling rates obtained in four different runs using a passive cryopreservation cooler comprising a cooling assembly in accordance with the present invention.

Detailed Description of An Embodiment

A preferred embodiment of the cooling assembly is shown in Figures 1a, b and Figure 2. Referring to Figures 1a,b and 2, the cooling assembly 10 comprises eighteen sample holders 12. The cooling assembly 10 is designed for use with a ten litre LN Dewar vessel.

Each sample holder 12 consists of a copper tube 114mm long, having an internal diameter of 3.8mm, sufficient to accommodate a conventional-size sample receptacle (a straw), which straw is 134mm long and has a volume of about 0.5ml (reference numeral 13, Figure 4). The eighteen sample holders 12 are arranged vertically in two concentric circles, the inner circle comprising six sample holders 12, the outer circle comprising twelve sample holders 12.

The sample holders 12 are retained by sample holder retaining means, which comprises a disk-like base plate 14 and a disk-like top plate 16, both 38mm in diameter. The

assembly 10 is also provided with a pair of support arms 18 which extend above the top plate 16 and then project outwards at right angles. The support arms 18 are 163mm long and the right-angled projection is 57mm long. The support arms 18 are welded at opposed positions on the circumference of the base plate 14 and the top plate 16.

The base plate 14 and the top plate 16 are both provided with an array of eighteen circular holes 20. Each hole 20 in the base plate 14 is associated with a respective sample holder and a respective sample receptacle support means 22. The receptacle support means 22 is made of PVC and comprises a projection 25 having at one end a tapered portion 24 and, at the other end, a flanged portion 26. The projection 25 is 8mm long. The tapered portion 24 is 2mm long and tapers from 4mm wide at its base to just 1.5mm wide at its apex. The flanged portion 26 is provided with an indent suitable for receiving the blade of a screwdriver. The diameter of the flanged portion 26 is greater than the diameter of the hole 20 in the base plate 14, such that the receptacle support means 22 can be secured by a screw thread engagement with the sample holder 12.

The hole 20 in the top plate 16 has a wide diameter at its lower surface, of appropriate dimension to accommodate the upper end of the sample holder 12. The hole 20 in the top plate 16 has a shoulder, at which point the diameter of the hole is reduced to 3.5mm, preventing passage of the sample holder 12 through the hole 20 in the top plate 16. However, the reduced diameter is still sufficiently large to allow passage therethrough of a typical 0.5ml straw.

Referring to Figure 1b and Figure 2, the top plate 16 and the base plate 14 both possess a circular central aperture 28, between which apertures 28 a channel is defined by the walls of the inner circle of sample holders 12. The channel thus formed through the cooling assembly 10 is wide enough to permit the passage therethrough of a sample receptacle.

Figure 1c illustrates the cooling assembly in use in conjunction with a passive cryopreservation cooler. The cooler comprises a Dewar vessel 30 containing a reservoir of LN (not shown). The Dewar vessel 30 has a neck region 31, the upper end of which

is defined by the opening of the vessel 30, the lower end being defined by a pronounced shoulder 33. The cooling assembly 10 is suspended in the neck region 31 of the vessel 30 by means of the support arms 18, which releasably engage an adjustable collar 32 placed around the outside of the opening of the vessel 30. Adjustment of the collar 32 can be performed to raise or lower the cooling assembly relative to the vessel 30. A different adjustment of the collar 32 varies the diameter thereof, such that the collar 32 can be fitted to vessels 30 with different size openings. LN vapour from the LN reservoir passes through the neck region of the vessel 30, thus acting to cool the cooling assembly 10 and the samples placed therein. The LN vapour is free to pass between the gaps between the sample holders 12, thus making immediate and intimate contact with every sample holder 12. This arrangement minimises variation between individual samples and allows for a substantially linear cooling rate.

When the samples have cooled to the desired temperature (which, with a substantially linear cooling rate, can be readily determined by measuring the duration of cooling), they are ready for storage. A storage canister 34 (Figure 1c) is rotated through 180°, thus being moved from the side of the vessel 30 to a position beneath the central aperture 28 in the cooling assembly 10. Straws may then be removed from the sample holders 12 and deposited through the channel defined in the assembly 10 and into the storage canister 34. When finished, the storage canister 34 is replaced at the side of the vessel 30. If desired, further straws can be placed in the cooling assembly 10 for cooling. When cooled, these further straws may be placed in the same or a different storage canister 34 (several such canisters 34 being provided within the vessel 30).

Figure 3 shows an exploded view of a slightly different embodiment of the cooling assembly 10. The assembly 10 comprises a disk-like sample holder alignment plate 35, which is positioned substantially mid-way along the sample holders 12 and serves to align the sample holders 12 between the corresponding holes 20 provided in the top plate 16 and the base plate 14. The alignment plate 35 is provided with holes 20 and a central aperture 28, corresponding to those in the top plate 16 and the base plate 14.

On each side of the central aperture 28 in the top plate 16 there is provided a small hole

36, which receives a corresponding pin provided on the underside of a disk-like adaptor plate 38. The adaptor plate 38 is essentially similar to the top plate 16, with an array of eighteen holes 20 and a central aperture 28 corresponding to those in the top plate 16. However, the holes 20 provided in the adaptor plate 38 are of smaller diameter than those in the top plate 16, and are intended to support the upper ends of 0.25ml volume straws, such that by adding or removing the adaptor plate 38 as appropriate, the cooling assembly 10 can be used for either 0.25 or 0.5ml volume straws as desired.

In use, the sample holders 12 are inserted at a slight angle into the holes 20 in the top plate 16, which allows sufficient clearance for the sample holders 12 to then be positioned vertically between the holes 20 in the top plate 16 and the corresponding holes 20 in the base plate 14. Each sample holder 12 is secured in position by insertion of a respective sample receptacle support means 22, which enters into a screw threaded engagement with a screw threaded portion provided at the lower end of the sample holder 12. A screw driver is preferably employed to tighten the engagement. The resulting cooling assembly 10 is substantially cylindrical.

Samples for cryopreservation are diluted with an appropriate cryodiluent (examples of which are well-known to those skilled in the art) and drawn into 0.25 or 0.5ml synthetic plastics straws. The straws are then sealed with colour-coded powder or beads and initially chilled: hydrated polyacrylamide gel pellets are frozen by immersion in LN and mixed with unfrozen gel pellets to form a gel/ice mixture. The gel/ice mixture is used for initial chilling of the straws. Referring to Figure 4, the straws 13 are then wiped dry and one straw placed in each sample holder 12. The tapered region 24 of the receptacle support means 22 becomes inserted a short distance into the lower end of the straw 13 and serves to prevent the straw from making substantial contact with the sample holder 12. Thus a thin air-jacket 15 is formed around each straw 13 within the sample holder 12, which air-jacket 15 inhibits the establishment of a temperature gradient vertically along the length of the straw 13 when the cooling process commences.

The straw 13 is supported at its upper end by passage through the hole 20 in the top plate 16. The sample holder 12 is of such a length as to ensure that a portion of the straw

projects above the top plate 16, which enables the straw to be grasped and facilitates retrieval of the straw from the cooling assembly 10.

Figure 5 shows an enlarged perspective view of a collar 32 for use in conjunction with the cooling assembly of the invention. The collar 32 is a substantially annular metallic ring, defining a central aperture of sufficient diameter to allow location of the collar around the outside of the opening of a Dewar vessel (such as 30 in Figure 1c). The lower surface of the collar 32 is provided with six identical slots 40 positioned at regular intervals around the circumference thereof. Each slot 40 accommodates the handle of a respective storage canister (34 in Figure 1c). The upper surface of the collar 32 is provided with three pairs of slots 42 positioned at regular intervals around the circumference thereof, each member of any pair of slots 42 being capable of accommodating the radially projecting portion of a respective cooling assembly support arm 18. Each pair of slots 42 is cut to a different depth, such that, by altering the slots 42 used to suspend the cooling assembly 10, the position of the cooling assembly 10 can be varied relative to the Dewar vessel 32.

Figure 6 shows three sets of graphs showing cooling rates. Figure 6a shows the graphs of temperature (20°C to -80°C) against time (minutes) obtained when cooling samples in a controllable rate cooler ("CRC"). As can be seen, the cooling rate is substantially linear between a temperature of 20°C and -50°C, with a minor departure from the straight line during freezing of the sample (at about -10°C). Figure 6c shows the graphs obtained using a conventional passive cooler. As can be readily seen, there is considerable variation between different samples, and the pre-freezing and post-freezing cooling rates are very different.

In contrast, Figure 6b shows the cooling rates obtained when using the cooling assembly of the invention in conjunction with a passive cooler. It can be observed that the cooling rate is substantially linear from 20°C to -50°C (as with the CRC) but declines slightly at lower temperatures. The only departure from the straight line in the temperature range 20 to -50°C is during freezing of the samples. The samples in question were samples of milt from the Tilapia *Oreochromis niloticus*.

Figure 7 shows similar cooling rates obtained with the same apparatus under field conditions in a hot country (Mexico). The mean pre-freezing cooling rate (of four runs) was 5.3°C/minute (SeM 0.4), and the mean post-freezing rate was 4.3°C/minute (SeM 0.25), giving an overall mean cooling rate of 4.8°C/minute (SeM 0.5). These values represent a substantially constant rate of cooling compared to cooling rates obtained with passive coolers without the cooling assembly of the invention. Further, the standard errors (SeM) are far lower, indicating much greater reproducibility than has previously been possible.

Clearly, embodiments other than those described above may be envisaged and are considered to fall within the scope of the invention. In particular, an embodiment is envisaged in which the cooling assembly is designed for use with a Dewar vessel of greater than ten litre capacity, the cooling assembly comprising seventy sample holders.

CLAIMS

1. A cooling assembly for use in a cryopreservation cooler, the assembly comprising: a plurality of individual sample holders, each sample holder being capable of accepting a sample receptacle containing a sample to be preserved; and sample holder retaining means for retaining the sample holders in a spaced apart relationship, such that a coolant fluid may pass between the sample holders.
2. A cooling assembly according to claim 1, comprising support means for suspending the assembly, in use, in the neck region of a cooler.
3. An assembly according to claim 2, wherein the assembly support means is capable of making releasable engagement with a collar provided on the cooler.
4. An assembly according to any one of claims 1, 2 or 3, wherein the sample holders, in use, are positioned substantially vertically.
5. An assembly according to any one of the previous claims, wherein the sample holders are substantially tubular.
6. An assembly according to any one of the previous claims, wherein the sample holders are made of material with a high thermal conductivity.
7. An assembly according to any one of the previous claims, wherein the sample holder retaining means comprises a base plate, said base plate comprising an array of holes or recesses, each hole or recess capable of accepting a respective sample holder.
8. An assembly according to any one of the previous claims, wherein the sample receptacles are supported by sample receptacle support means, said sample receptacle support means serving to prevent the sample receptacle from making substantial contact with the sample holder.

9. An assembly according to claim 8, wherein there is provided a plurality of sample receptacle supports, each such sample receptacle support being in screw threaded engagement with a respective sample holder.
10. An assembly according to any one of the preceding claims, wherein the receptacle support means comprises a tapered end portion which, in use, is inserted into the end region of a respective sample receptacle.
11. An assembly according to any one of the previous claims, wherein the assembly is formed with a channel therethrough, or forms such a channel in use, said channel being of sufficient dimension to permit the passage of a sample receptacle.
12. An assembly according to any one of the preceding claims, further comprising a sample receptacle delivery means locatable in a channel in the assembly, for guiding sample receptacles into a storage canister.
13. A cryopreservation cooler, comprising a cooling assembly in accordance with any one of claims 1 to 12.
14. A passive cooler according to claim 13, capable of achieving a substantially linear cooling rate in samples placed therein.
15. A cooler according to claim 13 or 14, comprising one or more storage canisters capable of accepting sample receptacles, said cooler being capable of performing simultaneously the functions of storage and cooling.
16. A cooler according to any one of claims 13, 14 or 15, comprising an adjustable collar capable of making a releasable engagement with an assembly support means provided on the cooling assembly.
17. A method of cooling samples prior to cryopreservation, comprising use of a cooling assembly in accordance with any one of claims 1 to 12.

18. A method of cooling samples according to claim 17, wherein the same cooler is used for both cooling and storage of samples, and wherein the cooling assembly does not require to be moved prior to placement of cooled samples in a storage canister within the cooler.

1/7

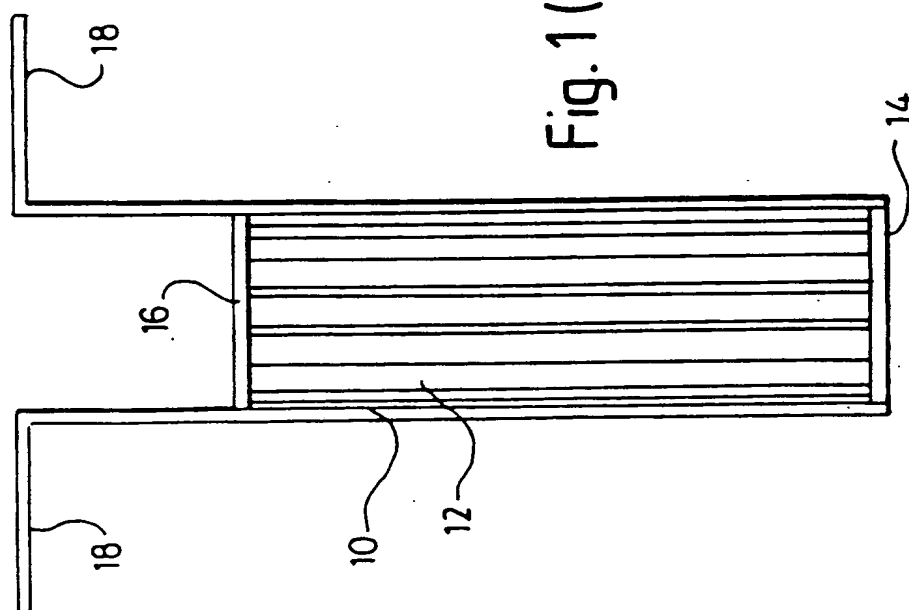


Fig. 1(a)

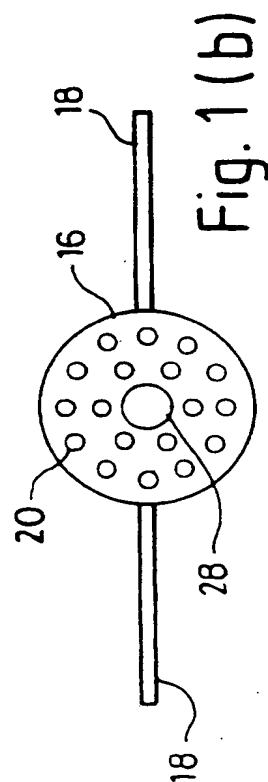


Fig. 1(b)

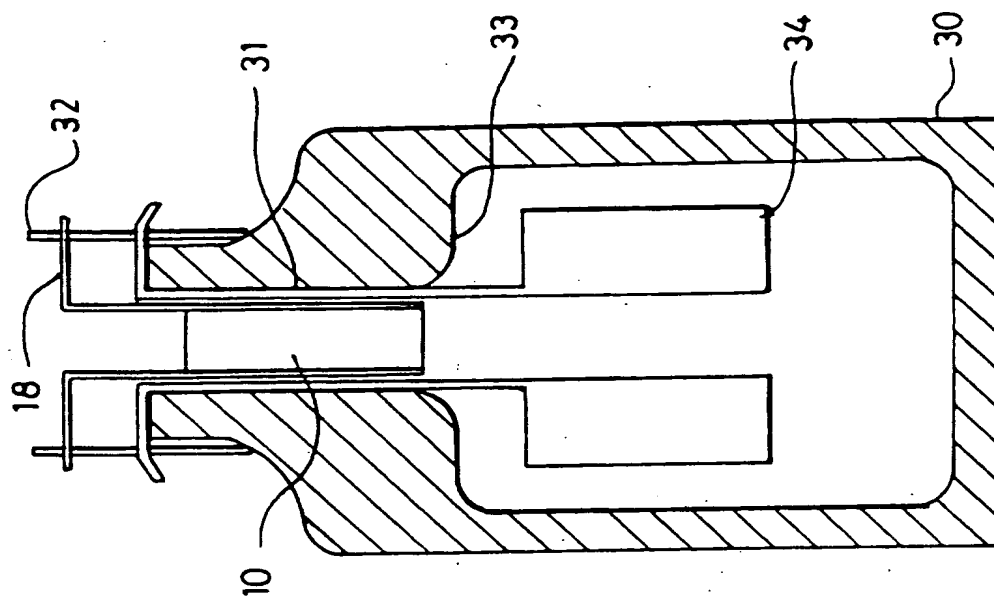


Fig. 1(c)

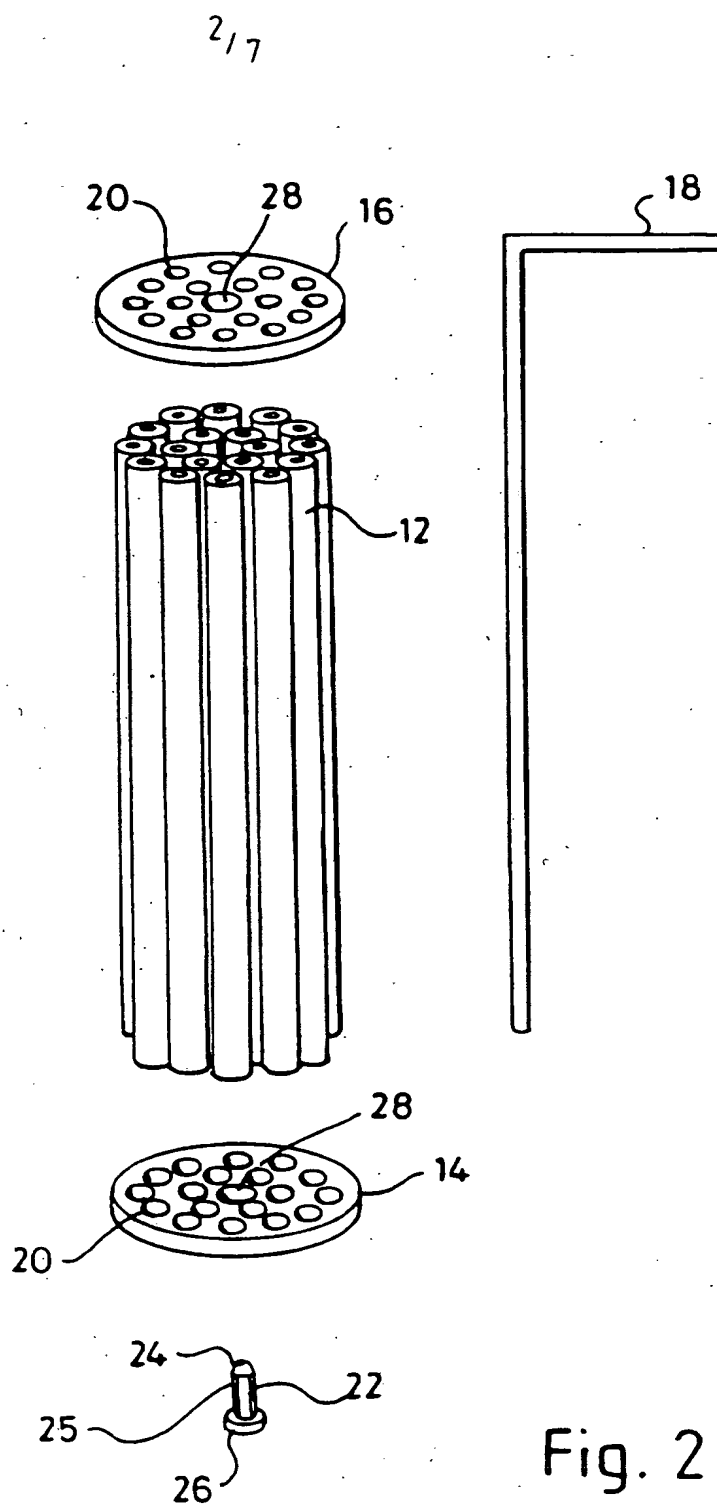


Fig. 2

3/7

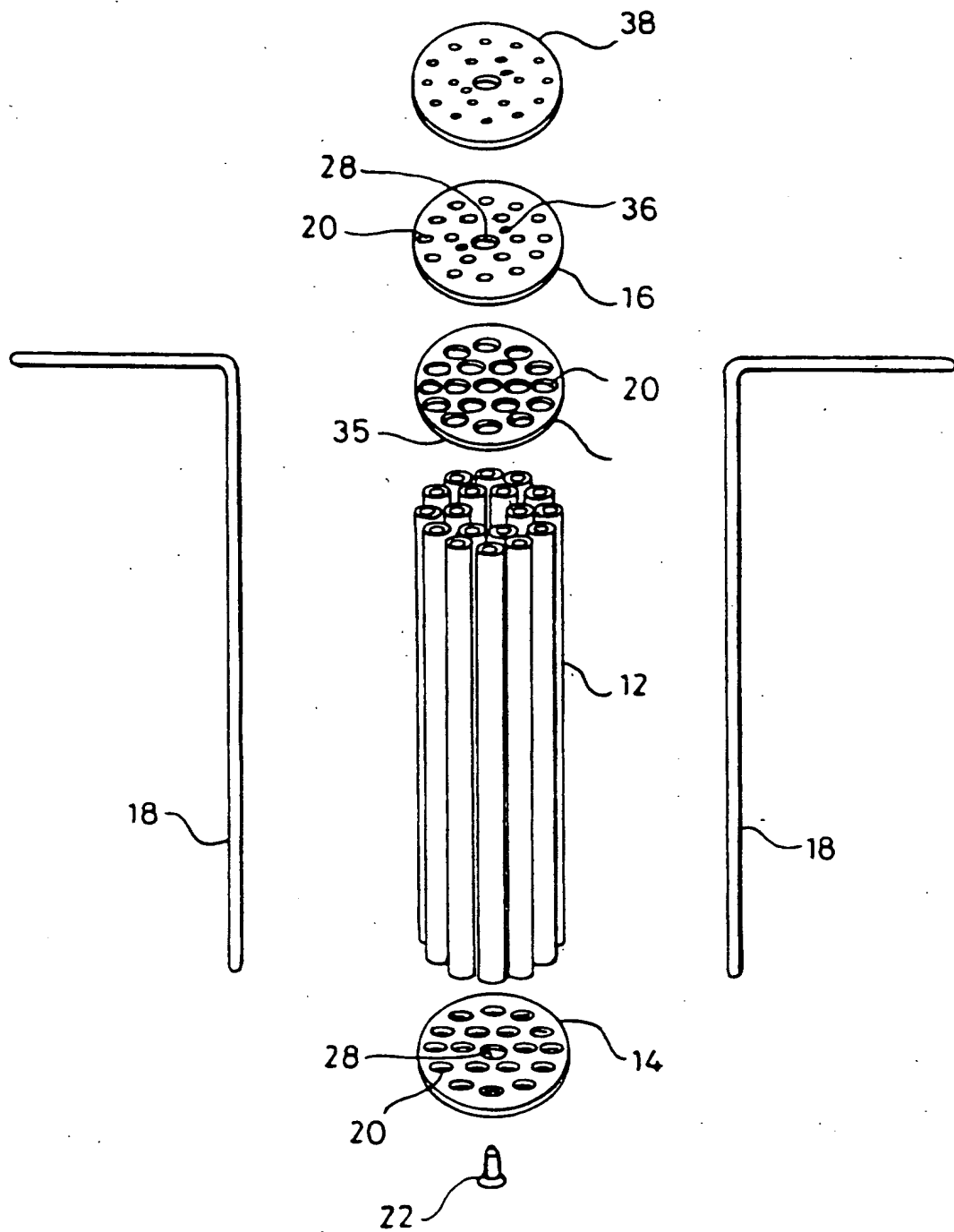


Fig. 3

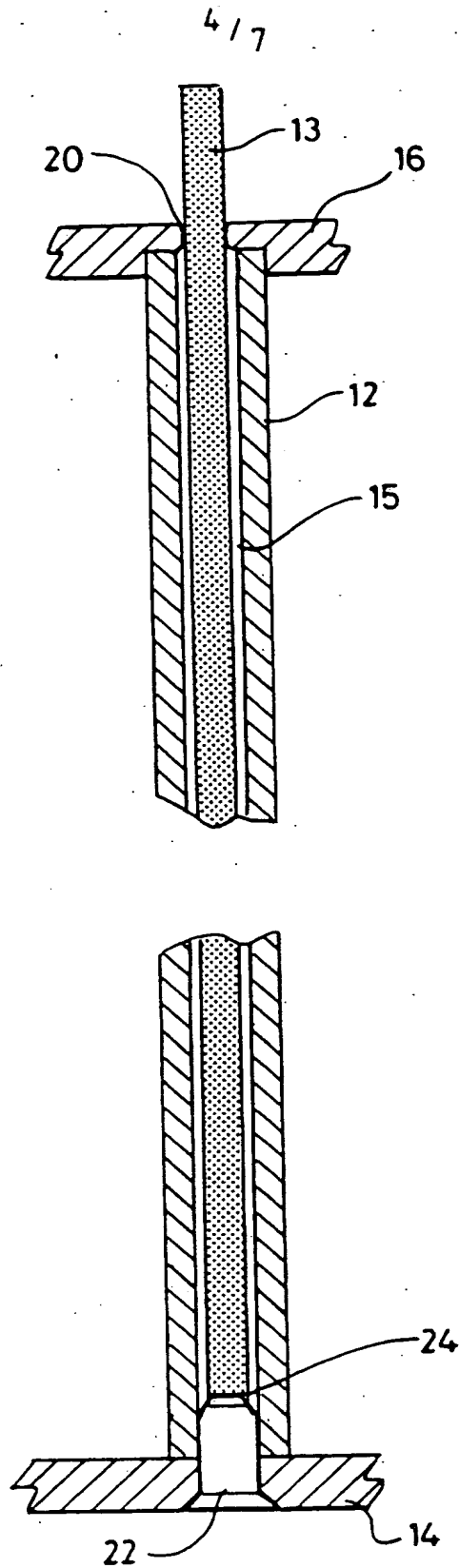


Fig. 4

5/7

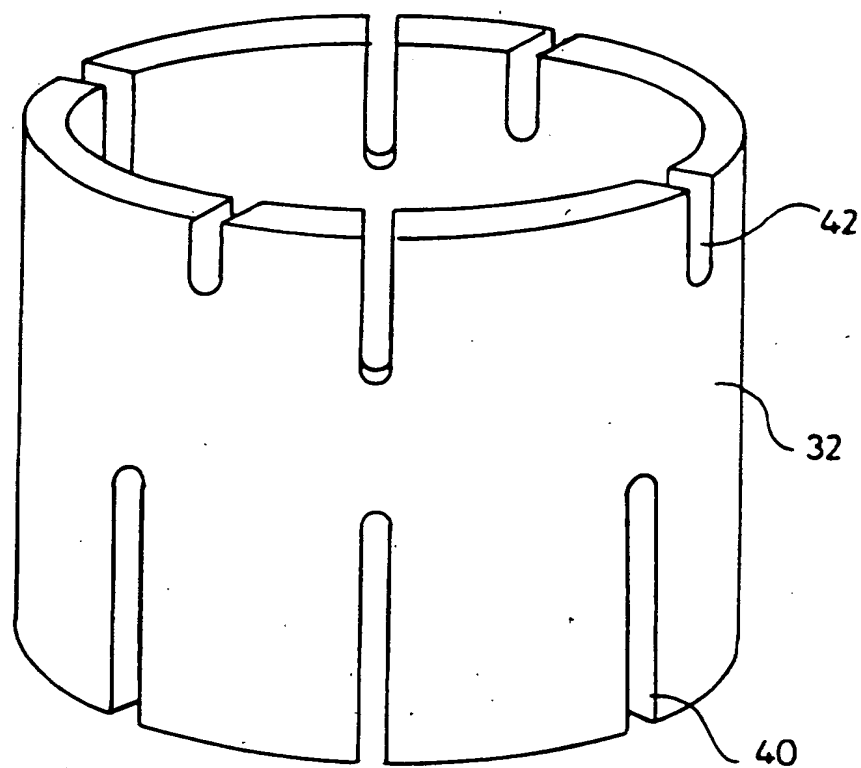


Fig. 5

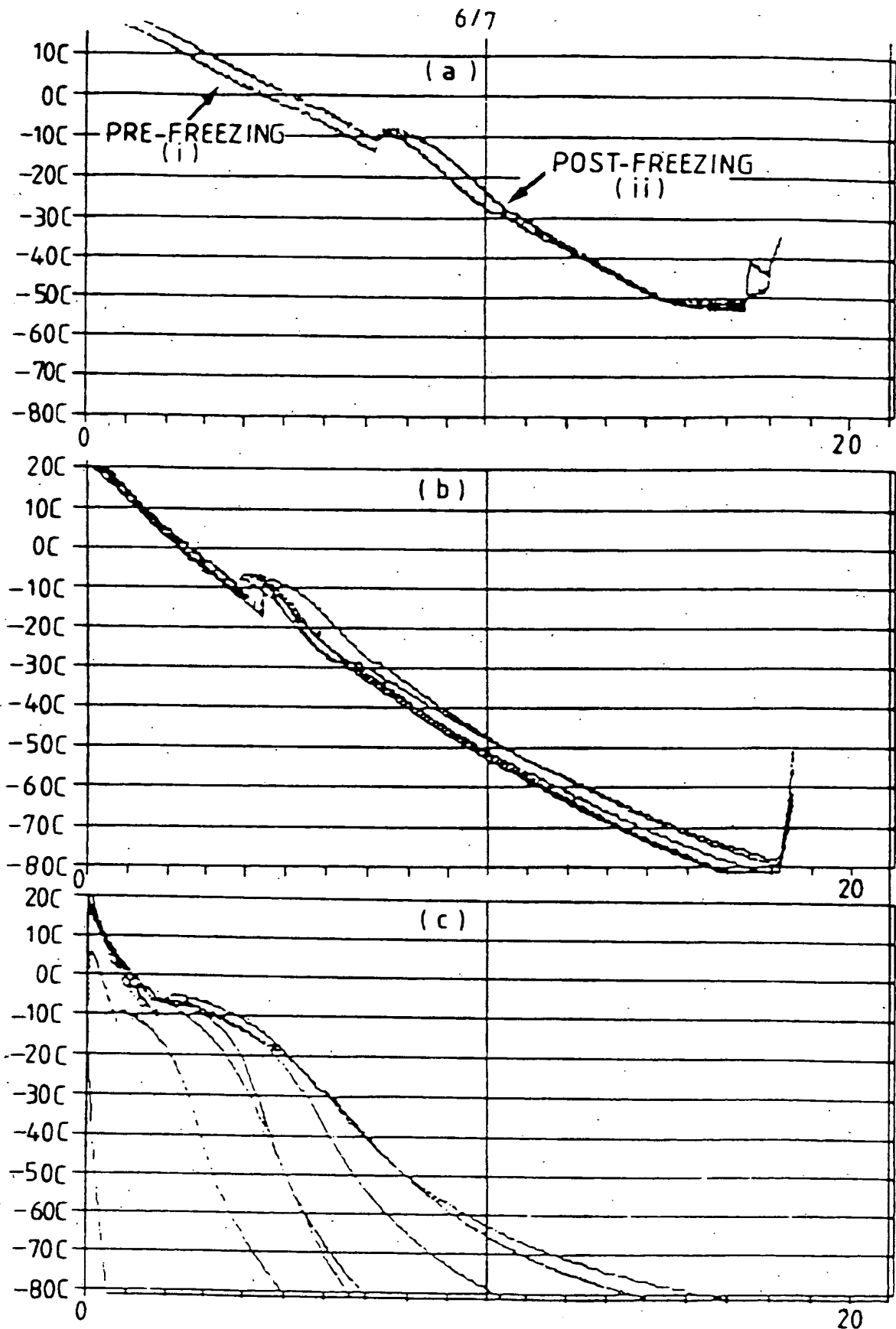


Fig. 6

7/7

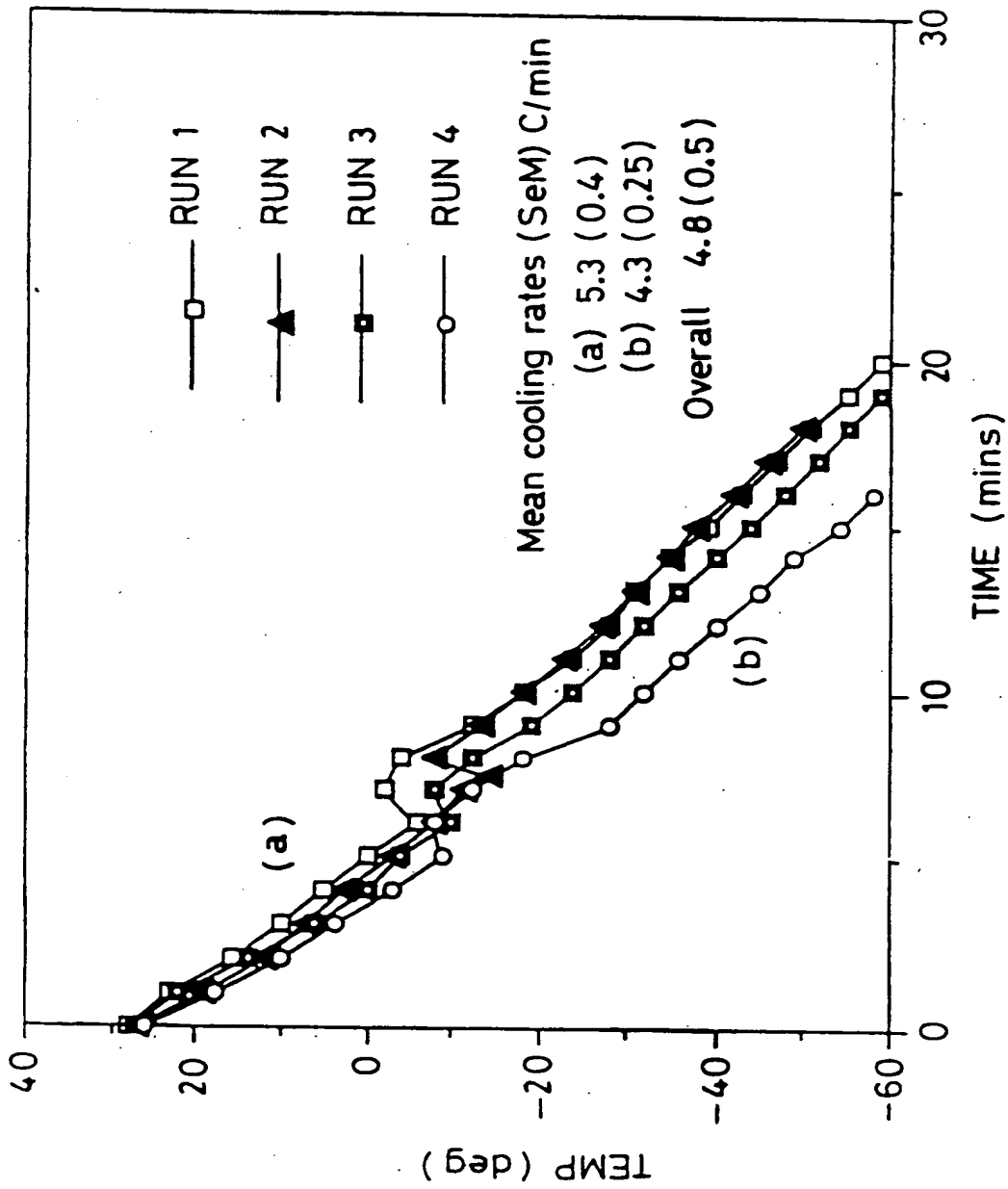


Fig. 7

FREEZE RUNS USING 10L DEWAR IN MEXICO

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 95/01677

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 F25D25/00 A01N1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 F25D G01N B01L A61J A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 757 692 (MCDONALD) 19 July 1988 see column 2, line 65 - column 5, line 8; figures 1-5 ---	1,2,4,5, 7,13,17
A	US,A,3 108 840 (CONRAD) 29 October 1963 see column 3, line 54 - column 6, line 48; figures 1-15 ---	1,4,5,7, 13,15, 17,18
A	EP,A,0 072 225 (HOXAN) 16 February 1983 see page 20, last paragraph - page 36, paragraph 1; figures 10-15 --- -/--	1,4,5,7, 13,17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *A* document member of the same patent family

Date of the actual completion of the international search

20 October 1995

Date of mailing of the international search report

25.10.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Boets, A

INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/GB 95/01677

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,3 707 079 (HAWKER) 26 December 1972 see column 3, line 19 - column 6, line 22; figures 1-3 ---	1,4,5,7, 13,17
A	US,A,3 942 668 (EBERLE) 9 March 1976 see column 2, line 16 - column 3, line 31; figures 1-6 ---	1,4,5,7, 13,17
A	EP,A,0 181 235 (FREEZE CONTROL) 14 May 1986 ---	
A	DE,A,17 51 667 (KARLSRUHER GLASTECHNISCHES WERK) 5 August 1971 ---	
A	GB,A,980 681 (CYROGENIC ENGINEERING) 20 January 1965 ---	
A	US,A,3 151 760 (COWLEY) 6 October 1964 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 95/01677

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4757692	19-07-88	NONE	
US-A-3108840	29-10-63	NONE	
EP-A-72225	16-02-83	JP-C- 1208000	29-05-84
		JP-A- 58027554	18-02-83
		JP-B- 58034135	25-07-83
		JP-C- 1208001	29-05-84
		JP-A- 58027555	18-02-83
		JP-B- 58034136	25-07-83
		JP-C- 1295459	26-12-85
		JP-A- 58086374	23-05-83
		JP-B- 60015858	22-04-85
		AU-B- 8670582	17-02-83
		CA-A- 1197467	03-12-85
		US-A- 4429542	07-02-84
		US-A- 4487033	11-12-84
US-A-3707079	26-12-72	NONE	
US-A-3942668	09-03-76	NONE	
EP-A-181235	14-05-86	AU-B- 577636	29-09-88
		AU-B- 4949785	15-05-86
		US-A- 4712607	15-12-87
DE-A-1751667	05-08-71	NONE	
GB-A-980681		NONE	
US-A-3151760	06-10-64	NONE	

This Page Blank (uspto)